a strong case cannot be made for S,N,O chelation by cysteine and penicillamine.

A substantial contribution of S,N chelation to kinetic Cu(II)–S bond stability is readily apparent from the k_2((Me$_6$tren)Cu–SR, (tmpa)Cu–S-pen) and k_3((tmpa)Cu–S-cys and –S-cme) rate constants. The presence of an optimal, five-membered S,N chelate unit certainly would stabilize the Cu(II) oxidation state and retard Cu(II)–S bond breaking in a reductive-elimination pathway leading to Cu(I) and a monodentate, N-bonded thiyl radical. Oxidation rate constants of S,N-bonded cysteine and its methyl ester are not greatly different in complexes with both (tmpa)Cu^{II} and (Me$_6$tren)Cu^{II}, but the reactivity of penicillamine is lower by more than 1 order of magnitude in both systems. This observation lends further support to a reductive-elimination mechanism requiring Cu(II)–S bond cleavage in the rate-determining step. Considering the surprisingly small rate of ring closure within (tmpa)Cu-NH$_2$-pen-S$^-$ and this reaction's ample thermodynamic driving force, even slower ring opening would be anticipated from microscopic reversibility considerations alone. The enhanced kinetic stability of Cu^{II}–S-pen certainly is not entirely related to S,N chelation, as shown most dramatically by the 30-fold difference in k_1(Cu(Me$_6$tren)$^{2+}$) for oxidation of cys-S$^-$ and pen-S$^-$.

Although stable five- or six-membered rings would not be readily formed from S-bonded glutathione (except through deprotonation of glycine or cysteine peptide nitrogens), the redox decay rate of (tmpa)Cu–S-glu is remarkably small and insensitive to pH. Extraordinarily large uncertainties in k_2, pK_{a1}, and pK_{a2} preclude a quantitative analysis of these parameters, but it is clear that the unusual relationship pK_{a1} > pK_{a2} must apply to achieve a good fit of the kinetic data to eq 3. In any case, the incorporation of cysteine into a protein environment seemingly is more important to Cu(II)–S bond stability than the reactions corresponding to the K_{a1}, K_{a2} ionizations. Partial encapsulation of the Cu(tmpa)$^{2+}$ unit by the polypeptide would hinder reductive elimination of thiyl sulfur in much the same way as chelation.

The consistently greater kinetic instability of (Me$_6$tren)Cu^{II}–SR adducts and the accessibility of only one kinetically relevant ionization in these complexes are the main points of contrast between the Cu(Me$_6$tren)$^{2+}$ and Cu(tmpa)$^{2+}$ kinetic results. The oxidizing strengths of the two Cu(II) centers appear to be similar, although a rigorous comparison is excluded by the irreversible cathodic wave of Cu(Me$_6$tren)$^{2+}$.[20] Steric interactions among the Me$_6$tren dimethylamino groups strongly hinder the rearrangement of Cu(Me$_6$tren)$^{2+}$ coordination geometry from trigonal bipyramidal toward square pyramidal or octahedral.[9,21] In contrast, four equatorial donor atoms are easily accommodated in complexes of tmpa and related polypyridylamine ligands.[10] For this reason, rearrangement of an *S*-thiolato, trigonal-bipyramidal (Me$_6$tren)Cu^{II}–SR complex into a six-coordinate thiolato, hydroxo species analogous to that proposed for the tmpa system (II) is unlikely, as is S,O chelation by the mercaptan. Considering the well-known high affinity of Cu(II) for nitrogen donor ligands,[22] the (Me$_6$tren)Cu–SR K_{a1} ionization most logically corresponds to S,N chelation of copper. Such chelation need not involve displacement of a Me$_6$tren dimethylamino group, as five-coordination could be retained by displacement of Cu(II) below the plane of these –N(CH$_3$)$_2$ donors.

Structural differences among the various mercaptide adducts of Cu(tmpa)$^{2+}$ and Cu(Me$_6$tren)$^{2+}$ complicate comparisons of rate constants between the two systems. Nevertheless, impressive relative stabilizations of the (tmpa)Cu^{II}–SR species by factors of ca. 10^4 and 10 are apparent at the low- and high-pH limits, respectively. Although increases in S(σ) → Cu(II) LMCT transition energies correlate in some instances with negatively tending $E°$(Cu(II,I)),[16] the (Me$_6$tren)Cu^{II}–SR rate data clearly show that such blue shifts do not necessarily result in greater *kinetic* stability of the Cu(II) oxidation state. Delocalization of thiolate sulfur negative charge over the tmpa pyridyl π systems could contribute to the reactivity difference between complexes with aliphatic and aromatic nitrogen donor atoms. Such delocalization would of course be analogous to that available through the imidazole group of physiological histidine ligands in the blue copper proteins.

Acknowledgment. Support of this research by the Robert A. Welch Foundation (Grant D-735) is gratefully acknowledged.

(20) Voltammograms generated from 1 mM Cu(Me$_6$tren)$^{2+}$ in pH 6.0, I = 0.1 M (MES) buffer showed a cathodic current maximum at –217 mV vs. NHE (50 mV/s sweep rate). $E°$(Cu(tmpa)$^{2+/+}$) is –147 mV under these conditions.[3] MES = morpholinoethanesulfonic acid.
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Electron Self-Exchange in Dicyanoiron Porphyrins

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Received August 15, 1984

Electron self-exchange rate constants have been measured for a series of dicyanoiron porphyrins by ^{1}H NMR. The rate constant for the Fe$^{II/III}$TPP(CN)$_2^{2-/-}$ system in Me$_2$SO-d_6 is 5.8 × 10^7 M^{-1} s^{-1} at 37 °C. Substituted tetraphenylporphyrins have slightly lower rate constants. Iron(II, III) protoporphyrin and deuteroporphyrin have self-exchange rate constants of ~1 × 10^7 M^{-1} s^{-1}. Assignments are given for the ^{1}H NMR resonances of the Fe(II) synthetic porphyrins and the ^{13}C resonances of CN$^-$ bound to Fe(II) porphyrins. The rate constants for cyanide exchange in the Fe(II) and Fe(III) systems are both <15 s^{-1}.

Introduction

The factors that control the rates of electron-transfer reactions of transition-metal complexes have been the focus of substantial interest.[1-3] In recent years this interest has been extended to biological systems.[4-6] One area of intense study has been the pathway of electron transfer in heme proteins.[7-9]

Most heme proteins have one edge of the heme exposed to solvent, and current thinking is that electron transfer generally takes place between the exposed heme edges of two proteins. The

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role of the protein in controlling electron transfer is still not well understood. Geometric, electronic, hydrogen-bonding, and π-electron cloud effects have been proposed. To understand the role of the protein in electron transfer, one needs to know the rate of electron exchange between two hemes free in solution. There have been only a few studies of this exchange.[10-12] These have measured the rate constant for electron exchange between the iron porphyrin and an organometallic or inorganic reagent and used the Marcus theory[3,13,14] to calculate the rate constant for iron porphyrin self-exchange. The rate constants calculated in this way range from $\sim 10^3$ to $\sim 10^{11}$ M^{-1} s^{-1}. It is important to know what factors cause this wide span. Possibilities include differences in heme, axial ligand, and solvent, as well as difficulties in determining redox potentials and self-exchange rates of the partners.

A technique that allows measurement of the self-exchange rates without these difficulties is that of NMR line broadening. In the NMR experiments, it is not necessary to know the properties of any other complex. In addition, the experiment can be run in organic solvents, so that it is unnecessary to use highly charged (hence water-soluble) hemes. We have used this technique to measure electron self-exchange in a series of iron porphyrins.[15,16]

In this paper we report the effects of changes in the porphyrin substituents on the self-exchange rate constants of dicyanoiron porphyrins. Both the effect of increasing steric bulk on the phenyl rings in tetraphenylporphyrin (TPP) derivatives and the difference between TPP and protoporphyrin derivatives have been studied. In addition, we discuss cyanide binding to both Fe(III) and Fe(II) porphyrins and assignments of NMR resonances in various Fe(II) complexes.

Experimental Section

Porphyrins were either purchased from Midcentury Chemicals or synthesized by pyrrole–benzaldehyde condensation in a propionic acid reflux.[17] Iron was inserted via the $FeCl_2$–DMF (TPP derivatives)[18] or $FeCl_2$–propionic acid (natural porphyrins)[19] procedures. Hemin purity was checked by TLC and by UV/vis and ^1H NMR spectroscopy. Palladium black (Alfa), $Na_2S_2O_4$ (Baker), D_2O (Aldrich), 40% NaOD in D_2O (Norell Chemical), and $Na^{13}CN$ (MSD Isotopes) were used as received.

^1H NMR Spectra. Proton NMR spectra were recorded on a JEOL FX-100 spectrometer operating at 99.54 MHz. The spectral width was 3–5 kHz. Typical spectra for the Fe(III) species, or exchanging mixtures, had 2–8K data points, an aquisition time of 0.4–1.3 s, and 100–500 scans. Line widths for the diamagnetic peaks were measured without any line broadening. Line widths for the paramagnetic peaks were measured with 5–10-Hz line broadening. The temperature of the probe was measured with a methanol thermometer.[20]

Sample Preparation and Data Collection. The heme (2.5–11 mM) was dissolved in Me_2SO-d_6 (MSD Isotopes, Merck) or CD_3OD (Merck) in a screw-top NMR tube (Wilmad). Oxygen was bubbled through the solution to prevent autoreduction. The stream of oxygen also helped effect solution, and the tube was usually bubbled for ≥30 min. The fully oxidized spectrum was then recorded. Nitrogen or argon was passed through the solution for 30 min, and autoreduction began. Spectra were taken every 20–60 min. Typical runs had 8–10 spectra; at least six were recorded in each case. Rate constants were calculated from the line broadening of the pyrrole resonance for the synthetic porphyrins and heme methyl resonances for the natural porphyrins.

Dissolution of the synthetic hemins at these concentrations is not entirely reproducible. In some runs it appeared that the hemin went into solution slowly over time. The area of the pyrrole peak in each spectrum was measured. Any run that showed a systematic increase in heme concentration of >10% was discarded. Attempts to dissolve the heme by heating, or by waiting for many hours, showed gradual heme destruction.

Reduction of the natural hemins was also effected either by adding aliquots of aqueous $Na_2S_2O_4$[21] or by adding aliquots of H_2 via a gas-tight syringe to a solution of the heme in Me_2SO-d_6 containing palladium black.[21b]

Data collection times were short (generally less than 45 s) to minimize peak broadening due to autoreduction. This was possible because the paramagnetic center induces a rapid relaxation of the protons. The spin–spin (T_2) and spin–lattice (T_1) relaxation times for pyrrole protons in low-spin Fe(III) hemins have been reported as $\simeq 10$ and $\simeq 13$ ms, respectively.[22] The solutions were pulsed rapidly, but with at least $5T_1$ between pulses. Autoreduction was usually constant over time and gave drifts of 0.3–1.0 Hz per peak. This drift value was subtracted from the measured peak width. In a few instances the rate of autoreduction increased with time. In these cases appropriate drift corrections were made for each spectrum.

The reactions in Me_2SO-d_6 were run in cyanide-saturated solutions. The CD_3OD solutions were 0.16 M in KCN. The solubilities of KCN in Me_2SO and CH_3OH at 25 °C are 0.12 and 0.31 M, respectively.[23] Solvent parameters used in the Marcus theory estimations (solubility, dielectric constant, etc.) are those of the protio solvents. The values may differ slightly from those of the deuterated solvents, but the differences are expected to be small, and no greater than those introduced by the presence of small amounts of water (i.e., addition of aqueous $Na_2S_2O_4$) to the solutions.

NMR Analysis. Exchange between a diamagnetic and spin $^1/_2$ paramagnetic site is a three-site-exchange problem, the unpaired electron with $m_s = \pm^1/_2$ exchanging with paired electrons. When the spin–lattice relaxation time of the electron (T_{1e}) is short compared to the lifetime τ for the exchange process, this reduces to a two-site problem.[24] This is the case for Fe(III) complexes, for which $T_{1e} \sim 5 \times 10^{-12}$ s.[25]

The observed line width for two species in fast exchange is the weighted average of the natural widths of A and B plus an additional broadening due to the chemical exchange of the two species[26]

$$1/T_2 = f_A/T_{2A} + f_B/T_{2B} + f_A f_B(\delta\omega)^2\tau$$

where f_A and f_B are the fractions of the mixture in sites A and B, T_{2A} and T_{2B} are the transverse relaxation times, $\delta\omega$ is the difference in frequency between unexchanging species, and $\tau = (1/\tau_A + 1/\tau_B)^{-1} = (k[B] + k[A])^{-1}$ is the lifetime.

Assuming that line broadening due to factors other than the intrinsic transverse relaxation rate is small (i.e., that $T_2 \simeq T_2^*$) and converting from rad/s (ω) to Hz (ν)[27]

$$W_{AB} = f_A W_A + f_B W_B + f_A f_B 4\pi(\delta\nu)^2/kc$$

where W_{AB}, W_A, and W_B are the peak widths at half-height for the exchanging peak and unexchanging peaks A and B, respectively.

The derivation of the line-width equations assumes that $2\pi(\delta\nu)\tau \ll 1$. For appropriate values of $\delta\nu$ the lower limits of measurable rate constants are 1×10^7 M^{-1} s^{-1} for the TPP derivatives and $\sim 5 \times 10^6$ M^{-1} s^{-1} for the natural porphyrins (c 0.01). The upper limits are given by the smallest line broadening that can be measured reliably, $\simeq 2$ Hz, for a k of $\sim 6 \times 10^8$ M^{-1} s^{-1} for the TPP derivatives (10% reduction, c 0.01) and $\sim 6 \times 10^7$ M^{-1} s^{-1} for the natural porphyrins (2% reduction, c 0.01). The complexity of the natural porphyrin spectra precludes more than a few percent reduction because the exchange-broadened lines begin to overlap.

Results

Autoreduction. Hexacoordinate ferric porphyrins bearing certain ligands can autoreduce in solution. The mechanism has

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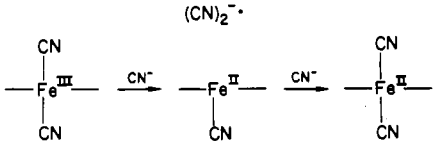
Table I. Electron Self-Exchange Rate Constants for Dicyanoiron Porphyrins in Me_2SO-d_6 at 37 ± 2 °C

porphyrin	rate const, 10^7 M^{-1} s^{-1}	porphyrin	rate const, 10^7 M^{-1} s^{-1}
TPP	5.8 ± 0.4	4-*i*-PrTPP[a]	3.1 ± 0.3
3-MeTPP	3.4 ± 0.3	DPDME[a]	1.0 ± 0.1
4-MeTPP	4.4 ± 0.6	PPDME[a]	1.5 ± 0.3
4-OMeTPP	2.9 ± 0.2	TPP[b]	1.6 ± 0.3

[a] At 30 ± 2 °C. [b] In CD_3OD at 37 °C.

been studied for cyanide, piperidine, and *N*-hydroxypiperidine as ligands and appears to involve an intramolecular electron transfer (which in the case of cyanide may be promoted by CN^-) to give the Fe(II) species and the ligand radical.[28,29]



For the dicyanohemins, a number of factors are known to affect the rate of autoreduction. Light increases the rate, although there is a dark pathway as well. In Me_2SO, increasing the concentration of cyanide or decreasing the concentration of water accelerates the rate of autoreduction. The rate also decreases as the porphyrin is made more basic.[28]

In this study the autoreduction rate constant was observed to decrease as major changes were made in the porphyrin structure [TPP derivatives > protoporphyrin IX dimethyl ester (PPDME) ≫ deuteroporphyrin IX dimethyl ester (DPDME) (no reduction)]. The time to reduce 10% of the hemin varied from ~2 to ~12 h. Most of the reactions were followed through 10–15% reduction, but some were taken to as high as 40% reduction; the electron-transfer rate constant determined did not depend on the extent of reduction. In most cases the autoreduction was linear with time. In a few cases autoreduction was observed to accelerate over time, but the electron-transfer rate constants measured in these runs did not differ significantly from those measured in runs where autoreduction was linear with time. The rate constants did not depend upon the concentration of iron porphyrin. Electron self-exchange rate constants are given in Table I.

There are two indications that the radicals produced in the autoreduction did not induce any artifacts in the electron-transfer rate measurements. First, the rates constants for electron transfer were independent of reducing agent in two sets of control experiments. In the first set $FePPDME(CN)_2^-$ was either allowed to autoreduce or reduced by aliquots of aqueous $Na_2S_2O_4$. In the second set $FeDPDME(CN)_2^-$ was either reduced by aliquots of aqueous dithionite or by the heterogeneous Pd/H_2 system. For both hemins, the rate constants measured for electron exchange were independent of the reducing agent. Second, in the autoreduction, the rate constants measured were independent of heme concentration and of the extent of the autoreduction. Both an increased heme concentration and an increased percentage of Fe(II) should have given an increase in concentration of radicals, but no effect on the rate constants was observed.

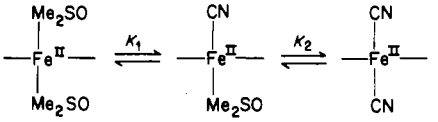
The NMR experiments do not show whether the $Fe^{II}P(CN)^-$ complexes have Me_2SO as a sixth ligand nor whether $Fe^{II}P(Me_2SO)_x$ has one or two Me_2SO molecules bound to the iron. The spectrum of $Fe^{II}TPP(Me_2SO)_x$ has been reported previously.[30] The complex must have at least one axial Me_2SO ligand because the spectra of $Fe^{II}TPP$ in benzene-d_6 (four-coordinate, spin 1)[31] and Me_2SO-d_6 are quite different. The ^1H NMR spectra of the ferric porphyrin monocyano complexes are consistent with assignments as low-spin species. They are presumably hexacoordinate, with Me_2SO in the sixth position. The $Fe^{III}P(Me_2SO)_x^+$ complexes are hexacoordinate, but high spin.[32]

Cyanide Equilibria. Both the natural and synthetic ferric porphyrins have high equilibrium constants for cyanide binding. For hemin concentrations in the range 1–10 mM, the dicyano complex is formed completely at [CN]/[hemin] ≥ 4.[33-35]

The ferrous porphyrins have much lower cyanide binding constants than the ferric porphyrins. However, for the tetraphenylporphyrin derivatives in KCN-saturated Me_2SO-d_6 the equilibrium constant for binding two cyanides was large enough that the dicyano species was formed. Autoreduction of the $Fe^{III}(CN)_2^-$ tetraphenylporphyrin derivatives gave spectra consistent with an $Fe^{II/III}(CN)_2^{2-/-}$ mixture in fast exchange. No additional peaks were seen, and thus no monocyano $Fe^{II}CN^-$ was present (see below). Complete reduction of the mixture with aqueous $Na_2S_2O_4$ gave only the dicyano ferrous $Fe^{II}(CN)_2^{2-}$ Similar results were obtained in CD_3OD.

The Fe(II) natural porphyrins have lower cyanide binding constants than the Fe(II) tetraphenylporphyrins. This is consistent with the greater basicity (more electron density on iron) of the natural porphyrins.[28] Reduction of the natural hemins protohemin dimethyl ester and deuterohemin dimethyl ester was achieved with H_2 and Pd black, with aqueous $Na_2S_2O_4$, and, in the case of protohemin, by allowing the solutions to autoreduce.

H_2 and Pd black reductions in Me_2SO solutions were run without the addition of any water. The reduction of deuterohemin in KCN-saturated Me_2SO gave only the monocyano species. The solubility of KCN in Me_2SO is 0.12 M,[23] indicating that for deuteroheme $K_2 < 1$ (assuming that ≥90% of the heme is the monocyano complex). This second Fe(II) equilibrium constant in Me_2SO is much lower than that of 2,4-dicysteine-substituted mesoporphyrin in water ($K_1 = 4.72 \times 10^5$ M^{-1} and $K_2 = 8.16 \times 10^3$ M^{-1} at 25 °C).[36] We attribute the difference to Me_2SO, which presumably binds more tightly than does water to the monocyanoheme.



Reduction of Me_2SO solutions saturated in KCN with aqueous $Na_2S_2O_4$ gave mixtures of the monocyano- and dicyanoiron(II) species. The percentages of the two species varied from experiment to experiment because the side reactions of $Na_2S_2O_4$ produce acid,[37] which protonates the CN^- ($pK_a = 9.4$).[38] Figure 1 shows a titration of a Me_2SO solution of $Fe^{II}DPDME$ with aqueous cyanide. In this experiment one can see all three species clearly. Integration of the spectra shows that the 2- and 4-H protons are to the high frequency side of the meso protons in the Me_2SO complex but that the positions are reversed in the monocyano and dicyano complexes.[35] The highest percentage of the dicyano ferrous complex was formed when basic aqueous $Na_2S_2O_4$ (a D_2O solution containing NaOD) was added to a Me_2SO solution of the heme. The base prevented protonation of the CN^-, and the

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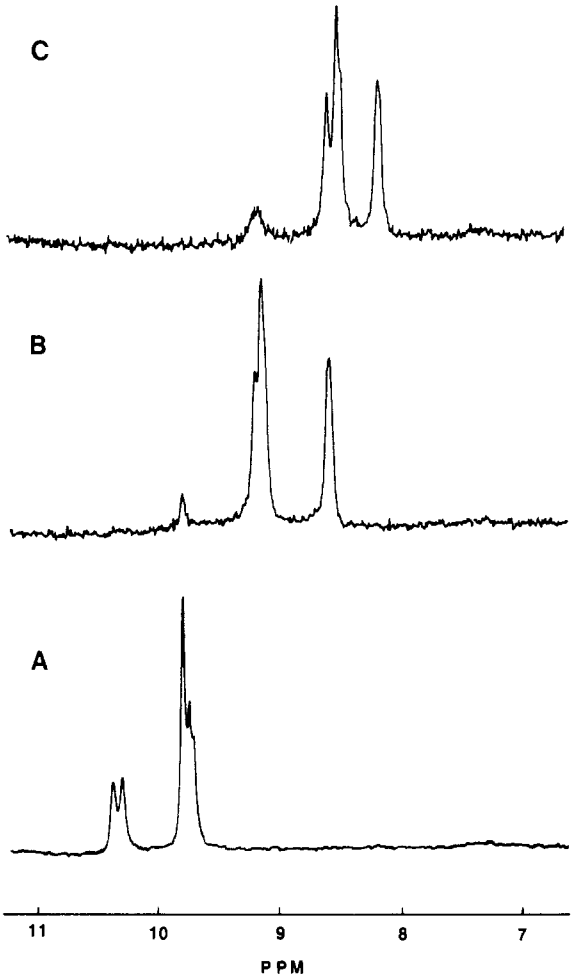


Figure 1. ^1H NMR of Fe(II) deuteroheme in Me_2SO as a function of added KCN (saturated solution in D_2O): (A) no added cyanide, Me_2SO complex; (B) ~6-equiv added cyanide, largely the monocyano complex; C) ~160-equiv added cyanide, largely the dicyano complex. See text for a description of experimental conditions and ^1H NMR assignments.

water apparently dissolved more cyanide.

The monocyano ferrous species can affect the measurement of the rate constant in two ways. If it does not participate in electron transfer, it merely changes the effective concentration of the iron porphyrin. If it does participate in electron transfer, a scheme more complicated than $Fe_a^{II}L_2 + Fe_b^{III}L_2 \rightleftharpoons Fe_a^{III}L_2 + Fe_b^{II}L_2$ is necessary. The former is the case, as seen in an experiment where deuterohemin was reduced with Pd/H_2. The deuterohemin system could be reduced up to 40% before the methyl peaks began to overlap enough to make line-width measurements inaccurate. As discussed above, the final reduced species was the monocyano complex. During the reduction two sets of peaks were seen (Figure 2). The broadening and shifting of the dicyano resonance indicated fast electron exchange between the $Fe^{II}(CN)_2^{2-}$ and $Fe^{III}(CN)_2^-$ species. The $Fe^{II}(CN)^-$ peaks were not broadened, indicating that this complex was not undergoing electron exchange with $Fe^{III}(CN)_2^-$ on the NMR time scale. No conclusion can be drawn regarding the rate constant for electron self-exchange between the monocyano $Fe^{II}CN^-$ and $Fe^{III}CN$ because there was essentially no $Fe^{III}CN$. For deuterohemin, the total concentration of exchanging heme, c, was calculated from the integrals of the two sets of resonances.

For the protoporphyrin system, reduction was only taken to ~3% (autoreduction of ~3 h). Beyond this point, the broadened ring methyl resonances began to overlap, and measurement of the line widths was difficult. Thus, although final reduction gave a mixture of the $Fe^{II}(CN)^-$ and $Fe^{II}(CN)_2^{2-}$ species, at the percentages of reduction used to calculate the rate constant (≤3%), only a small amount of the heme would have been the monocyano species and no correction was necessary to the total concentration

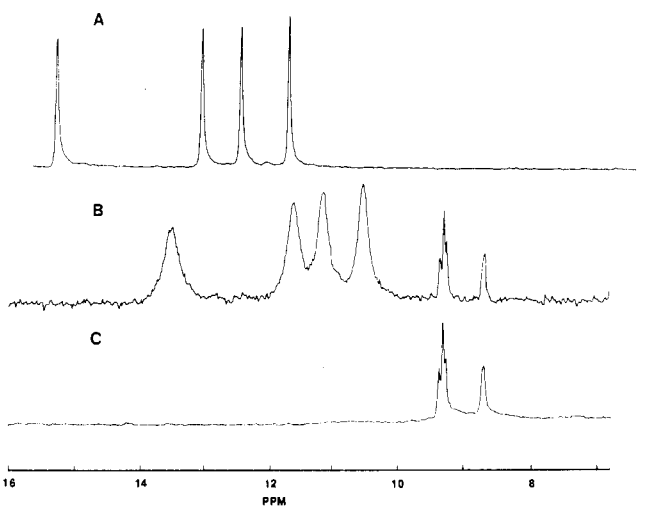


Figure 2. Electron exchange in a mixture of $FeDPDME(CN)_2$ and FeDPDME(CN): (A) $Fe^{III}DPDME(CN)_2^-$; (B) $Fe^{II/III}DPDME(CN)_2^{2-/-}$ (four broad methyl resonances at higher frequency) and $Fe^{II}DPDME(CN)^-$ (meso protons at 9.3 and 2,4-H at 8.7 ppm); (C) $Fe^{II}DPDME(CN)^-$. The vertical scales are arbitrary.

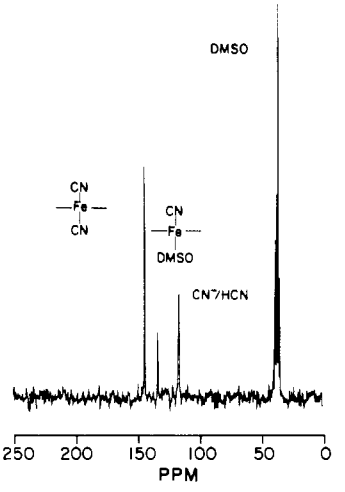


Figure 3. ^{13}C NMR spectrum of 12 mM $Fe^{II}TPPCl$ with 4 equiv of $Na^{13}CN$ in Me_2SO-d_6 [Fe(II) reduced with saturated aqueous $Na_2S_2O_4$].

of exchanging iron porphyrin, c. Rate constants are given in Table I.

^{13}C NMR of CN Bound to Fe(II) Hemes. When $Fe^{III}TPP(^{13}CN)_2^-$ in Me_2SO was reduced with aqueous $Na_2S_2O_4$, the ^{13}C NMR spectrum showed three resonances (Figure 3). The intensity of the peaks as a function of $[Na^{13}CN]$ allowed assignment of the resonance at 148 ppm to the dicyano complex and that at 137 ppm to the monocyano complex. The difference in chemical shift between these two resonances is consistent with the 10 ppm chemical shift difference between ferrocyanide (177 ppm)[39] and the trans CN of its Me_2SO complex (167 ppm).[40] The third resonance was broad ($\Delta\nu_{1/2} > 40$ Hz) and varied in position from 114 to 134 ppm from run to run. This is attributed to a fast exchange between HCN (110.9 ppm)[41] and free CN^- (168.6 ppm) as described by Wang et al.[33] The variation of $[H^+]$ from run to run in our solution is due to differing amounts of acid produced in side reactions of $Na_2S_2O_4$.[37]

The natural porphyrins showed similar patterns. A solution of $Fe^{II}DPDME$ in Me_2SO saturated with CN^- (~20 equiv) and containing a small amount of NaOD showed three peaks at 140.0

(39) Pesek, J. J.; Mason, W. R. *Inorg. Chem.* **1979**, *18*, 924–928.
(40) Malin, J. M.; Schmidt, C. F.; Toma, H. E. *Inorg. Chem.* **1975**, *14*, 2924–2928.
(41) Olah, G. A.; Kiovsky, T. E. *J. Am. Chem. Soc.* **1968**, *90*, 4666–4672.

Table II. $Fe^{II}(4\text{-}OCH_3TPP)L_2$ Chemical Shifts[a] in $Me_2SO\text{-}d_6$

	ligand		
reson	$(CN)_2$	$(CN)(Me_2SO)$	$(Me_2SO)_2$
pyrrole	7.92	8.31	11.6[b]
meta[c]	7.82	7.93 7.84	8.07[d]
ortho[c]	7.19	7.26[e]	7.36[d]
OCH_3	3.96	4.00	4.04

[a] All shifts referenced against $Me_2SO\text{-}d_5$ (2.5 ppm). [b] $\nu_{1/2} = 25$ Hz. [c] $J_{o,m} = 8.5$ Hz. [d] Assignments may be reversed. [e] Center of multiplet.

Table III. Chemical Shifts of Heme Resonances in Diamagnetic Dicyanoiron(II) Tetraphenylporphyrin Complexes[a]

heme	β-H	ortho H	meta H	para H
TPP	7.89	7.64	7.60[b]	7.60[b]
3-MeTPP	7.89	7.70	7.51[b] 2.51 (CH_3)	7.41[b]
4-MeTPP	7.90	7.79 (d, $J = 8.1$ Hz)	7.42 (d, $J = 8.1$ Hz)	2.55 (CH_3)
4-OMeTPP	7.92	7.82 (d, $J = 8.5$ Hz)	7.19 (d, $J = 8.5$ Hz)	3.96 (CH_3)
4-*i*-PrTPP	7.90	7.80[c]	7.51 (d, $J = 8.6$ Hz)	1.45 (d, CH_3, $J = 7.4$ Hz)

[a] In $Me_2SO\text{-}d_6$. [b] Highest of a multiplet. [c] Presumably a doublet with second peak under pyrrole resonance.

(monocyano), 149.0 (dicyano), and 154 ppm ($HCN \rightleftharpoons H^+ + CN^-$). Another sample with a $[CN^-]/[heme]$ ratio of 7.4 and no NaOD also showed three resonances, at 127.8 ($HCN \rightleftharpoons H^+ + CN^-$), 140.7, and 148.4 ppm. The monocyano species showed an increased line width of the bound CN^- in the latter experiment (5.8 vs. 9.2 Hz), which may represent acid-catalyzed promotion of cyanide exchange. This has been observed for cyanide exchange in ferrocyanide.[42]

^1H NMR Assignments in Fe(II) Synthetic Hemes. Paramagnetic Fe(III) hemins have been studied extensively by NMR, but there have been relatively few studies on the diamagnetic Fe(II) hemes. In this work it was important to assign the resonances in the Fe(II) and Fe(III) dicyano and monocyano species to ensure that electron exchange was occurring only between the dicyano species. For the synthetic hemes, this proved easiest in the 4-OMeTPP system.

Replacement of the 4-H in $Fe^{III}TPP$ by 4-OMe simplified the aromatic region of the spectrum both by eliminating the para H resonance and by increasing the chemical shift difference between the ortho and meta resonances. Addition of 1.9 equiv of $Na^{13}CN$ to $Fe^{III}(4\text{-}OMeTPP)Cl$ in Me_2SO (2.1 mmol heme, 3.9 mmol CN^-) gave a ferric heme spectrum with two pyrrole ^1H resonances, one at −15.12 ppm (dicyano) and a second at −17.0 ppm (monocyano). The former constituted >90% of the mixture.

Reduction of this solution with aqueous dithionite gave a mixture of Fe(II) monocyano and Me_2SO species. ^{13}C NMR showed the monocyano complex (137.1 ppm) but no dicyano complex (147.6 ppm). ^1H NMR showed the monocyano (pyrrole 8.31 ppm) and Me_2SO (pyrrole 11.7 ppm) complexes. Irradiation experiments and preparation of $Fe^{II}(4\text{-}OMeTPP)(Me_2SO)_x$ led to the assignments in Table II.

Assignments for the dicyano iron(II) synthetic porphyrins are given in Table III. In Fe(II)/Fe(III) mixtures each porphyrin resonance appears only once, because the complexes are in fast exchange. Therefore, assignments in the Fe(III) complexes, and (linear) plots of the chemical shift vs. percent reduction in Fe(II)/Fe(III) mixtures, allowed assignment of the resonances in the Fe(II) complexes.

Cyanide Exchange Rate. In cytochromes, the protein amino acid side chain fixes the two axial ligands in place. In the model system, the ligands are free to dissociate (Scheme I), and other electron-transfer pathways besides the desired one (e.g., between two five-coordinate or between a five-coordinate and a six-coordinate heme) are possible. In cyanide-saturated Me_2SO, the synthetic Fe(II) and Fe(III) porphyrins are generally both found as the dicyano species, with the exceptions discussed above.

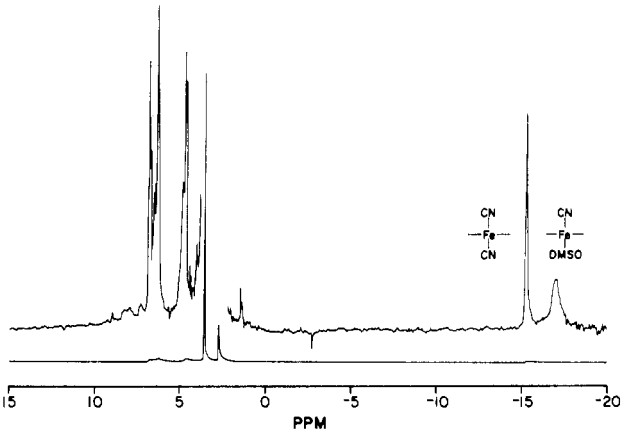
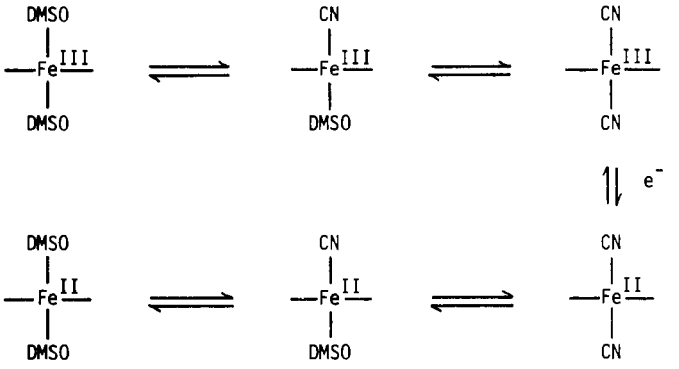


Figure 4. ^1H NMR spectrum of 10 mM $Fe^{III}TPPCl$ and 1.2 equiv of $Na^{13}CN$ in $Me_2SO\text{-}d_6$.

Scheme I



It is not only the equilibria, but the rate constants in the scheme that are of concern, however. Even if ligand exchange does not lead to species that can transfer an electron easily, the ligand-exchange process can broaden the NMR resonances. It was thus necessary to examine the cyanide exchange rates of the Fe(II) and Fe(III) hemes.

The axial ligand exchange rate constant for the dicyano complex of $Fe^{III}TPP$ was probed by titration of a solution of $Fe^{III}TPP$ in Me_2SO with $Na^{13}CN$ Addition of less than 2 equiv of $^{13}CN^-$ produced two upfield pyrrole resonances: the dicyano (−15.6 ppm, $\Delta\nu_{1/2} = 10$ Hz) and the monocyano (−17.3 ppm, $\Delta\nu_{1/2} = 54$ Hz) species (Figure 4).[43] With further addition of cyanide the dicyano/monocyano ratio increased correspondingly. The line width of the dicyano species did not vary with the dicyano/monocyano ratio, indicating that ligand exchange is slow. Detection of a 2-Hz broadening would have given an exchange rate constant of ≈ 6 s^{-1}, which establishes the upper limit.

Two other studies have led to similar conclusions. Wang et al. looked at the ^1H NMR of ferriprotoporphyrin cyanide–Me_2SO solutions and concluded that the rate for cyanide exchange was slower than 160 s^{-1} at 65 °C.[39] Goff estimated an exchange rate of less than 170 s^{-1} at 96 °C from ^{13}C data.[44] Assuming an activation energy of 20 kcal mol^{-1} for ligand exchange, he calculated a lifetime at 25 °C of seconds or longer.

Ferrous porphyrin cyanide exchange was studied with $Fe^{II}TPP(CN)_2^{2-}$ generated by the autoreduction of an argon-

(42) Chadwick, B. M.; Sharpe, A. G. *Adv. Inorg. Radiochem.* **1966**, *8*, 83–176.

(43) The breadth of the pyrrole resonance of the $Fe^{III}TPP(CN)$ complex may indicate Me_2SO exchange between $Fe^{III}TPP(CN)$ and $Fe^{III}TPP(CN)(Me_2SO)$ complexes.

(44) Goff, H. *J. Am. Chem. Soc.* **1977**, *99*, 7723–7725.

Table IV. Literature Electron Self-Exchange Rate Constants for Iron Porphyrins Calculated from Cross Reactions by Using the Marcus Theory

porphyrin[a]	reductant	temp, °C	μ, M	pH	k_{11}, M^{-1} s^{-1}	ref
$FeTMPyP(H_2O)(OH)^{4+/3+}$	$Ru(NH_3)_6^{2+}$	25	0.05	2-4	$>1 \times 10^9$	10
$FeTMPyP(H_2O)^{5+/4+}$	$Ru(NH_3)_6^{2+}$	25	0.05	2-4	1.2×10^6	10
$FeTMPyP(Im)_2^{5+/4+}$	$Ru(NH_3)_6^{2+}$	25	0.5	4.5	$>10^7$	10
$FeTPPS(H_2O)^{5-/4-}$	$V(H_2O)_6^{2+}$	25	0.25	2	$\sim 1 \times 10^3$	11
$FePPIX(CN)_2^{3-/4-}$ [b]	$SO_2^{-}\cdot$	25	0.5	*c*	8×10^{10}	12a
$FePPIX(H_2O)^{-/2-}$ [b]	$SO_2^{-}\cdot$	25	0.1[d]	7-9	7×10^5	12

[a] Abbreviations: TMPyP, tetrakis(4-*N*-methylpyridyl)porphyrin; TPPS, tetrakis(4-benzenesulfonato)porphyrin; PPIX, protoporphyrin IX. [b] Charge assumes both propionic acids fully ionized under the experimental conditions. [c] 10^{-2} M NaOH. [d] 2% sodium dodecyl sulfate.

purged solution of $Fe^{III}TPPCl$ in Me_2SO containing an excess of $^{13}CN^-$. The bound cyanide resonance was observed at 147.8 ppm ($\Delta\nu_{1/2}$ = 7 Hz). If we assume that the unexchanging line width equals that of ferrocyanide (3 Hz),[40] then the ligand preexchange lifetime is 0.08 s at 30 °C. This is a lower limit; a larger unexchanging line width would give a longer lifetime.

The lifetimes of the $Fe^{II}(CN)_2^{2-}$ and $Fe^{III}TPP(CN)_2^-$ complexes are at least 0.08 and 0.2 s, respectively. The preexchange lifetime of these species in electron transfer is only 10^{-5} s. Cyanide exchange is therefore very slow with respect to electron transfer.

Aggregation. Many metalloporphyrins dimerize or aggregate in solution;[45] this was not a problem in this study, however. The extent and type of aggregation depend upon the metal ion, porphyrin substituents, axial ligands, solvent, and counterion. In general, *meso*-tetraphenyl-substituted low-spin porphyrins do not aggregate appreciably.[46] In particular for this study, the ^1H NMR spectra of low-spin dicyano ferric TPP derivatives are not concentration dependent from 0.001 to 0.020 M in Me_2SO-d_6 or CD_3OD.[47] Aggregation is more of a problem for natural porphyrins. However, Viscio and La Mar found no concentration dependence in the spectra of low-spin dicyanohemins in CD_2Cl_2 or CD_3OD at room temperature.[48] Similarly, we have found that the position and line widths of the dicyano complex of Fe(II) deuteroheme dimethyl ester are independent of heme concentration between 1 and 5 mM at 30 °C.

Discussion

Electron-Exchange Rate Constants Calculated from Cross Reactions. Historically, self-exchange rate constants for hemes have been calculated from cross-reaction experiments by using the Marcus equation:[2,3,13,14]

$$k_{12} = (k_{11}k_{22}Kf)^{1/2} \quad (1)$$

$$\ln f = (\ln K)^2/4(\ln (k_{11}k_{12}/Z^2)) \quad (2)$$

where k_{12} is the rate constant for electron transfer between a heme and another redox partner, k_{22} is the electron-exchange rate constant of the partner, K is the equilibrium constant for electron transfer, f is a correction factor (generally close to 1, Z is the collision frequency), and k_{11} is the desired self-exchange rate constant for the heme. This approach has been used extensively in heme protein chemistry[49] but has seen limited application in heme chemistry itself.

Rate constants for hemes calculated in this way are given in Table IV. They span a wide range, from $\sim 10^3$ to $\sim 10^{11}$ M^{-1} s^{-1}. It does appear that the (high-spin) five-coordinate complexes transfer electrons more slowly than the (low-spin) six-coordinate complexes. This is in accord with the general observation that changes in geometry slow electron-transfer reactions and that rate constants fall in the order low spin–low spin > high spin/high spin >> high spin/low spin.[50-52] It also appears that the $FeTMPyP(H_2O)^{5+/4+}$ system has a much larger self-exchange rate constant than the $FeTPPS(H_2O)^{3-/4-}$ system (1.2×10^6 and $\sim 1 \times 10^3$ M^{-1} s^{-1}, respectively). A similar situation is found in the cobalt porphyrins, where $CoTMPyP(H_2O)^{5+/4+}$ and $CoTPPS(H_2O)_2^{3-/4-}$ have rate constants of 20 and 6.1×10^{-2} M^{-1} s^{-1}, respectively.[11,53]

These self-exchange studies are complicated by a number of factors, however. In water, a number of heme species can be found. This is particularly true for the $FeTMPyP(H_2O)$ system; this complex has been the focus of recent studies.[10,54] In systems using $SO_2^{-}\cdot$ as a reductant, eq 1 is not useful because the $SO_2^{-}\cdot/SO_2$ self-exchange rate is not known. The exchange rate constants were calculated on the basis of the $SO_2^{-}\cdot/Co^{III}TMPyP(H_2O)_2$ reaction. The difficulty with this can be seen in the $FePPIX(CN)_2^{3-/4-}$ self-exchange rate constant, which is approximately 1 order of magnitude greater than diffusion control. Determination of electron self-exchange rates by NMR removes many of the difficulties in the cross-reaction approach, especially measurements of the self-exchange rate constant of the reductant and of the redox potentials of both species.

Table I shows that substituents in the meta and para positions on the phenyl ring slow the rate of electron self-exchange only slightly. In terms of the Marcus theory, the rate constant for self-exchange can be expressed as[13,14]

$$k = \kappa Z \exp(-\Delta G^*/RT) \quad (3)$$

$$\Delta G^* = \Delta G^*_{in} + \Delta G_{out} + w_r \quad (4)$$

where κ is a probability factor (set equal to 1) and Z is the collision frequency ($\sim 10^{11}$ M^{-1} s^{-1}). The inner-sphere reorganization energy ΔG^*_{in} is given by

$$\Delta G^*_{in} = \frac{1}{4} \sum_i \frac{f_1 f_2}{f_1 + f_2} (\Delta a^0)_i^2 \quad (5)$$

where f_1 and f_2 are the force constants of the ith bond in the two reactants, Δa^0 is the difference in equilibrium bond distance between reactant and product, and the summation is over all the

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(46) Snyder, R. V.; La Mar, G. N. *J. Am. Chem. Soc.* **1977**, *99*, 7178–7184.
(47) La Mar, G. N.; Del Gaudio, J.; Frye, J. S. *Biochim. Biophys. Acta* **1977**, *498*, 422–435.
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(49) Wherland, S.; Gray, H. *Biol. Aspects Inorg. Chem.*, [*Symp.*] **1978**, 289–368.
(50) The importance of the change in geometry has been shown recently in a study of the heterogeneous electron-transfer rates constants of $Fe^{III}OEP$ and -TPP complexes bearing two substituted pyridines.[51] No substantial changes were observed in the rate constants as a function of spin state. However, the X-ray structures of $Fe^{III}OEP(3\text{-}Cl(pyr))_2$ at 98 K (predominately low spin) and 293 K (thermal mixture of high- and low-spin states) show that no motion of the metal atom is required in the spin state transition.[52] This indicates that the rate of electron transfer is more closely related to movement of the iron atom than the spin state change per se.
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intramolecular vibrations. The outer-sphere reorganization energy, ΔG^*_{out}, is

$$\Delta G^*_{out} = \frac{1}{4}\left(\frac{e^2}{2r}\right)\left(\frac{1}{D_o} - \frac{1}{D_s}\right) \quad (6)$$

where r is the radius of the reactant ion, D_o is the optical dielectric constant (the square of the refractive index), and D_s is the static dielectric constant. The work term may be evaluated according to the Debye–Hückel theory:

$$w_r = z_1 z_2 e^2 / 2rD_s(1 + 2\beta r\mu^{1/2}) \quad (7)$$

where z_i are the charges on the reactants, e is the charge on the electron, μ is the ionic strength, and $\beta = (8\pi Ne^2/1000D_s kT)^{1/2}$.

For the porphyrin complexes ΔG^*_{in} is small (<1 kcal) because the structure of the complex changes little on going from Fe(II) to Fe(III).[55] ΔG^*_{out} is calculated according to eq 6 where $r = (r_1 r_2 r_3)^{1/3}$ and the r_i are the radii along the perpendicular axes. For $K[Fe(TPP)(CN)_2]$, $r = 6.2$ Å.[56] In Me_2SO, $1/D_o - 1/D_s = 0.437$ and therefore $\Delta G^*_{out} = 2.9$ kcal/mol. In methanol, $\Delta G^*_{out} = 3.6$ kcal/mol. The Coulombic interaction energy, expressed by eq 7, is 0.4 kcal mol^{-1} in Me_2SO ($\beta = 0.418$ Å^{-1} M$^{-1/2}$ at 37 °C) and 0.5 kcal mol^{-1} in MeOH ($\beta = 0.499$ Å^{-1} M$^{-1/2}$ at 37 °C). Thus, in these systems the activation energy for electron transfer is due mainly to outer-sphere reorganization. The differences in $(\Delta G^*_{out} + w_r)$ between Me_2SO and MeOH is 0.8 kcal. At 37 °C, the reaction is predicted to be approximately 4 times faster in Me_2SO than in MeOH, as was found.

Increased steric bulk on the heme will increase r and decrease both ΔG^*_{out} and w_r. This should give a higher electron self-exchange rate constant.[3,57] Instead, the rate constant is slightly lower. This may be explained as a decrease in orbital overlap between the complexes, resulting in a reaction with a lower probability factor κ. Similar steric effects have been found in the electron self-exchange rate constants of iron phenanthroline complexes[27] and in cross reactions of $Ru(NH_3)_5(py)^{3+/2+}$ complexes with $Co(1,10\text{-phen})_3^{3+/2+}$.[58] Other studies have found either no steric effects or somewhat complicated patterns.[59] In our study steric effects are small, and even a change in macrocyle from tetraphenylporphyrin to the natural protoporphyrin skeleton produces at most a factor of 5 change in the electron self-exchange rate constant. This indicates that electron transfer in low-spin hemes that are not highly charged is relatively insensitive to the exact nature of the macrocycle.

Electron self-exchange rate constants in $FeP(CN)_2^{2-/-}$ (this work) and $Fe(TPP)(RIm)_2^{0/+}$ complexes[15,16] (10^7–10^8 M^{-1} s^{-1}) are only slightly faster than those in the small cytochromes (10^6–10^7 M^{-1} s^{-1}).[15,16] This observation is somewhat surprising; it might have been expected that the proteins would transfer electrons more slowly because most of the heme is covered by the amino acid chain.[60] The difference could be explained in terms of the Marcus theory if w_r and ΔG^*_{out} for proteins were very small. Wherland and Gray have calculated w_r for a number of cytochromes; the values are $0.1 < w_r < 1.0$ kcal.[49] It is difficult to estimate ΔG^*_{out} in proteins because amino acid residues nearby the heme are not free to reorient; it is possible that ΔG^*_{out} is very small. Part of the similarity between the models and proteins may then be explained on the basis of decreases in heme exposure, w_r, and ΔG^*_{out} in the proteins.

However, the large rate constants for electron transfer in small cytochromes may also be a function of factors not considered explicitly in eq 3–7. Possibilities include specific interactions of residues between two proteins, orientation of the proteins in one another's electric field during approach, and formation of complexes. These also may help to explain the wide range of electron self-exchange rate constants, 10^2–10^7 M^{-1} s^{-1}, that have been measured in the heme proteins themselves.

Acknowledgment. We thank the National Institutes of Health (Grants AM 30479 and BRSG S07 RR07054) for support of this work.

Registry No. $Fe^{III}TPP(CN)_2$, 40988-77-0; Fe^{III}(3-MeTPP)$(CN)_2$, 63871-86-3; Fe^{III}(4-MeTPP)$(CN)_2$, 63871-85-2; Fe^{III}(4-MeOTPP)$(CN)_2$, 94929-68-7; Fe^{III}(4-*i*-PrTPP)$(CN)_2$, 94943-94-9; Fe^{III}DPDME$(CN)_2$, 59006-49-4; Fe^{III}PPDME$(CN)_2$, 64060-98-6; Fe^{II}(4-OMeTPP)(CN)(Me_2SO), 94929-69-8; Fe^{II}(4-OMeTPP)$(Me_2SO)_2$, 94929-70-1; $Fe^{II}TPP(CN)_2$, 64060-99-7; Fe^{II}(3-MeTPP)$(CN)_2$, 94929-71-2; Fe^{II}(4-MeTPP)$(CN)_2$, 94929-72-3; Fe^{II}(4-OMeTPP)$(CN)_2$, 94929-73-4; Fe^{II}(4-*i*-PrTPP)$(CN)_2$, 94929-74-5.

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Kinetics of Oxidation of Cuprous Complexes of Substituted Phenanthroline and 2,2′-Bipyridyl by Molecular Oxygen and by Hydrogen Peroxide in Aqueous Solution

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Received September 14, 1984

The kinetics and the reaction mechanism of copper(I) complexes of 5-methyl-1,10-phenanthroline, 5-chloro-1,10-phenanthroline, 5-nitro-1,10-phenanthroline, 2,9-dimethyl-1,10-phenanthroline, and 2,2′-bipyridyl with oxygen and hydrogen peroxide have been investigated in aqueous solutions with use of the pulse radiolysis technique. The oxidation by O_2 is second order in the copper(I) complex, while the oxidation by H_2O_2 is first order in the copper(I) complex. Both reactions are first order in oxidants. The kinetic results of the oxidation of copper(I) complexes by oxygen are interpreted by a mechanism that proceeds via a superoxide intermediate.

Introduction

Recently, it has been demonstrated that degradation of double-stranded DNA by 1,10-phenanthroline (op) requires the presence of copper salt, a reducing agent, and O_2.[1-5] The degradation is always inhibited by catalase and in some cases by superoxide dismutase (SOD), suggesting the involvement of H_2O_2 and O_2^-, respectively, in the process.[1-3] The degradation is also

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0020-1669/85/1324-1087$01.50/0